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Relationship Between Extractive and Durability of Six Species of Wood

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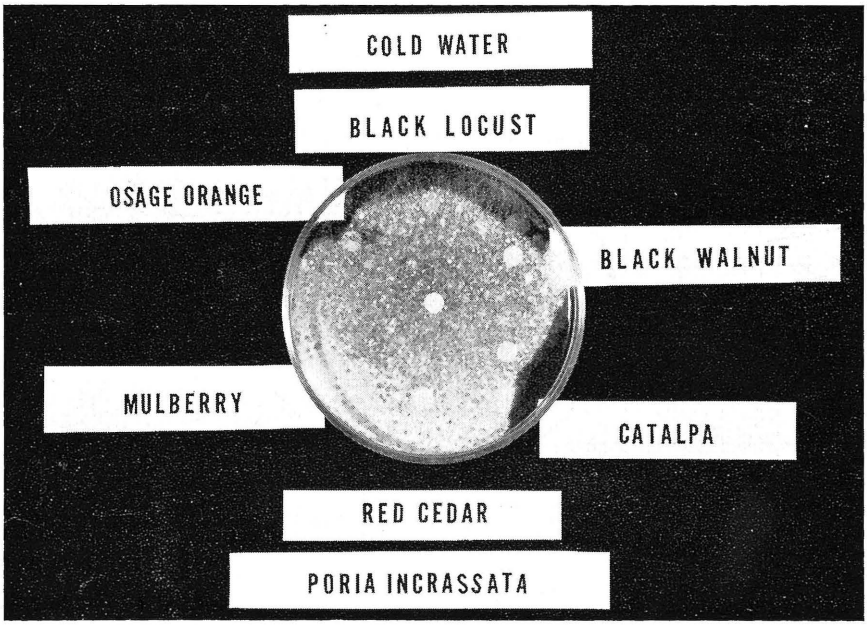

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ON THE COVER:

Fig. 2.—Cold water—no inhibition

RELATIONSHIP BETWEEN EXTRACTIVE AND DURABILITY OF SIX SPECIES OF WOOD

WAYNE K. MURPHEY

INTRODUCTION

The Ohio Agricultural Experiment Station has been conducting research concerning the natural durability of wood for 50 years. The object of the previous tests was to determine the relative service life of fence posts. In order to identify the compound or compounds imparting this durability a series of experiments with black locust (Robinia pseudoacacia), black walnut (Juglans nigra), catalpa (Catalpa speciosa), eastern redcedar (Juniperus virginiana), mulberry (Morus rubra), and osageorange (Maclura pomifera) was initiated. These species had been found to have relatively long service life as fence posts. The project has been divided into two phases. The initial phase concerned with determining the solvents which might extract a durable substance is reported in this paper. The second phase will attempt to classify the portion of the extract which imparts the durability.

Previous investigations concerned with natural durability of wood have attempted to correlate specific gravity, age of trees, and chemical composition of the cells. Scheffer and Englerth (1952) found no practical significance between size and age of second-growth Douglas Fir. Suolahti (1948) concluded decay resistance is not related to site classes, specific gravity or amount of late wood.

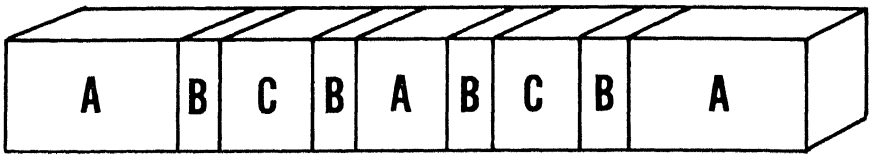
Extractives of naturally durable species have been shown to impart resistance to decay fungi. Several compounds have been extracted which have anti-fungal properties. Barnes and Gerber (1955) extracted a compound-tetrahychoxystibene from the heartwood of osageorange which was resistant to wood destroying fungi.

Rennerfelt (1943) names two compounds (pinosylvin and monomethylether) responsible for the durability of Scotch pine. Zabel (1948) concluded that tannins were toxic to wood-rotting fungi. Most of the compounds found to inhibit fungi have been aqueous extracts.

This experiment was initiated to test the decay inhibitants in the species previously listed by extraction in cold water, hot water, alcohol, benzene, alcohol and a combination of benzene-alcohol, alcohol and hot water.

Method and Material

The wood was obtained from trees cut from the Secrest Arboretum at the Ohio Agricultural Experiment Station. Two trees of each species were cut into 4′ bolts. The second 4-foot bolt was ripped into 2″ × 2″ sticks. Since Scheffer (1943) found that decay resistance in oak is highest in the upper part of the trunk and the outer heartwood is more resistant than that near the pith, the 2″ × 4″ sticks from which the samples were taken were those nearest, but not including, the sapwood. Care was taken to eliminate the "heartwood-sapwood" zone in eastern redcedar. Two sticks from each selected bolt were then divided lengthwise as shown in Figure 1.



A—discarded
B—specific gravity
C—extraction sample

Fig. 1.—Disposition of blocks cut from 2″ ×2″ sticks.

The blocks (C) to be extracted were split, ground in a Wiley mill and sieved. The material which passed through a 60-mesh screen but was retained on a 80-mesh screen was kept for extraction. All of this size material from each species was mixed together prior to removal of forty eight-gram samples used in individual solvent extractions. Twelve, two-gram samples were extracted with each of seven solvents or solvent combinations. (The remaining twenty-four grams were used in moisture content and ash percentage determinations.) Benzene-alcohol, alcohol, acetone-ether extractions of each species were accomplished, using the procedure (for benzene-alcohol extraction) outlined by American Society for Testing Materials (7). The standard procedures for cold water and complete extraction were also followed. The

hot water extractables were removed using the Soxhlet extraction apparatus instead of that outlined by A.S.T.M. The results of statistical analysis showed no significant difference at the 0.1 percent level between the two methods. The Soxhlet method provided an easier means of handling materials with less variation around the mean.

Moisture content and percentage ash determinations were in accordance with A.S.T.M. recommended procedures.

Exchange water was used throughout this study. Spectrographic analysis showed amounts of zinc, nickel and copper in the available distilled water. Since this might have effects on the spectrographic analysis, the distilled water was discarded.

All extracts were evaporated to dryness by lyophilization except the ether extracts which were evaporated at 60° F. The dry residue from complete (benzene-alcohol; alcohol; hot water) extracts was dissolved in exchange water to a volume of 250 ml. The cold water, hot water, benzene-alcohol, acetone, ether and alcohol residues were dissolved in exchange water to a volume of 100 ml.

The material used for determining the percentage of extractables removed was ashed and a spectrographic analysis made to determine amounts of inorganic elements removed by extraction. Three non-durable species: Corsican pine, river birch, and white ash were also ashed to provide comparisons of inorganic constituents between durable and non-durable woods.

All weights were determined on a 100 gram capacity Mettler Gramatic balance to 0.0001 grams. Constant weights were recorded when the material varied less than $\pm$ 0.0002 grams.

The specific gravity of each bolt was obtained to insure uniformity of the bolts. Although no correlation has been found between durability and specific gravity by other investigators, Englerth and Scheffer (1954) suggest it may be important due to the presence and amounts of chemicals which may be reflected in specific gravity.

The following fungi used to test the extractives were obtained from the Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

94042-R—*Coniophora puteana* (Fr.) Karst., rot isolate from black cherry, Westline, Kane, Pennsylvania, collected by W. A. Campbell and R. W. Davidson, September 12, 1940.

Madison 617—*Lenzites trabea* Pers. ex Fr., isolate from western redcedar, Madison, Wisconsin, 1926. Test isolate of the Forest Products Laboratory.

90876-R—*Merulius lacrymans* Wulf. ex Fr., rot isolate from *Quercus* board, Asheville, North Carolina, collected by C. Hartley, October 1946.

72074—*Polyporus versicolor* L. ex Fr., isolate from cankered area on beech, Vermont, collected by W. A. Campbell, July 1938. Madison number 697, test isolate of the U. S. Forest Products Laboratory.

Madison 563—*Poria incrassata* (Berk. & Curt.) Burt, isolate from southern yellow pine, Griffinsburg, Virginia, 1926. Test isolate of the U. S. Forest Products Laboratory.

94267—*Poria monticola* Murr., rot isolate from Douglas Fir boat timbers, Annapolis, Maryland, collected by C. Hartley and R. W. Davidson, December 1941. Madison number 698, test isolate of the U. S. Forest Products Laboratory.

To test the inhibition of the extractive on the fungi, three techniques were developed. The first technique consisted of placing 1 milliliter of the concentrated extract on a piece of 9 cm. diameter No. 1 Whatman filter paper in a petri dish. The carrier was evaporated and five milliliters of potato-dextrose agar medium slanted on the impregnated paper. The plate was then inoculated and incubated for three weeks at laboratory temperature of approximately 83° F. The dishes were placed in polyethylene bags to restrict loss of moisture. After incubation the plates were rated as to absence or presence of fungal growth on the agar slant, and on the paper. Controls were made by placing 1 ml. of the solvent on filter and handling them the same way as the extract impregnated paper.

In the second technique, 10 ml. of the extract was evaporated on filter paper, from which ¼" discs were punched. This permitted the testing of the inhibitory properties of all species extracted with a given solvent on a single plate. Ten milliliters of potato-dextrose agar were poured into sterilized petri dishes, allowed to harden and the dishes were placed in a polyethylene bag. Ten milliliters of propylene oxide were evaporated in the bag containing the plates, the bag sealed and stored for 24 hours. The plates were removed from the bag and aerated for

three days before inoculation. A 5 mm. inoculum slurry of fungus, agar, and deionized water was injected into a single dish. After a 10-day incubation period the plates were analyzed for the absence or presence of fungal growth.

A third technique differed only in placing a piece of the growing fungus in the center of the plate under a three week incubation period before analyzing the plates. Although this method required a longer period of incubation, sterile conditions were more easily maintained than in techniques No. 1 and No. 2.

RESULTS AND DISCUSSION

The results of the slurry technique tests are shown in Table 1. Cold water, acetane and ether extractions are omitted from Table 1, since no fungistatal properties were shown by these solvents. The number indicates the count of positive inhibition for each species and fungus.

Appearance of the inoculated plates after 10 days using the slurry technique with Poria incrassata as the test fungus is shown in Figure 2

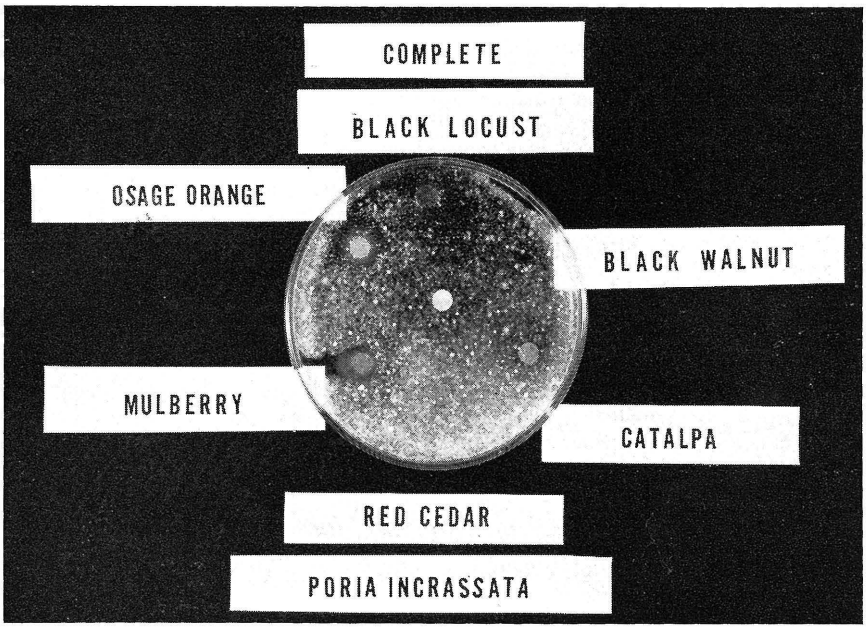


Fig. 3.—Complete extracts of osageorange and mulberry show inhibition of fungus.

TABLE 1.—Number of Replications Showing Inhibition of Extractives of Six Species by Six Fungi Using the Slurry Technique

Species		Solvents			
		Hot Water	Alcohol	Benzene: Alcohol	Complete
Black locust	Cp(1)	3	--	--	--
	Lt	3	--	--	--
	Ml	3	--	--	--
	Pi	3	--	--	--
	Pm	3	--	--	--
	Pv	3	--	--	--
Black walnut	Cp	1	--	2	--
	Lt	1	--	3	--
	Ml	1	--	3	--
	Pi	--	--	3	--
	Pm	--	--	3	--
	Pv	--	1	--	--
Catalpa	Cp	1	--	1	--
	Lt	--	--	3	--
	Ml	1	--	2	--
	Pi	--	2	3	--
	Pm	1	1	3	--
	Pv	--	1	2	--
Eastern redcedar	Cp	--	2	--	--
	Lt	3	--	--	--
	Ml	--	--	--	--
	Pi	--	--	--	--
	Pm	--	3	--	--
	Pv	--	--	--	--
Mulberry	Cp	2	3	3	2
	Lt	3	3	3	2
	Ml	2	2	2	2
	Pi	3	3	3	3
	Pm	3	3	3	3
	Pv	1	2	2	1
Osageorange	Cp	--	3	3	3
	Lt	1	3	3	2
	Ml	--	3	3	2
	Pi	--	3	3	3
	Pm	1	3	2	3
	Pv	--	3	3	1

Cp(1)—Coniophora puteana
Lt —Lenzites trabea
Ml —Merulius lacrymans
Pv —Polyporus versicolor
Pi —Poria incrassata
Pm—Poria monticola

Numbers represent replications in which inhibition occurred

through 6. The filter paper disc has been impregnated with the extractive of the indicated species. The center disc is a control soaked in the solvent used for extraction.

Each series was replicated three times and only those tests which showed positive inhibition are counted. The results of the first and third techniques agree with those shown in the table. The first technique was more difficult to read due to contamination. The results of the third technique varied slightly because the fungal mat grew from the center. It was difficult to define areas of inhibition in the agar since some fungi bridged the impregnated disc. Pictorially the results of the slurry technique were superior to the other two. It also required a shorter period between plate inoculation and reading.

The plates were read as to presence or absence of growth and not to degree of inhibition. Some migration from the filter paper took place, thus the extract front became larger and attempts to classify each extract as to degree of inhibition seemed unnecessary. Those extracts

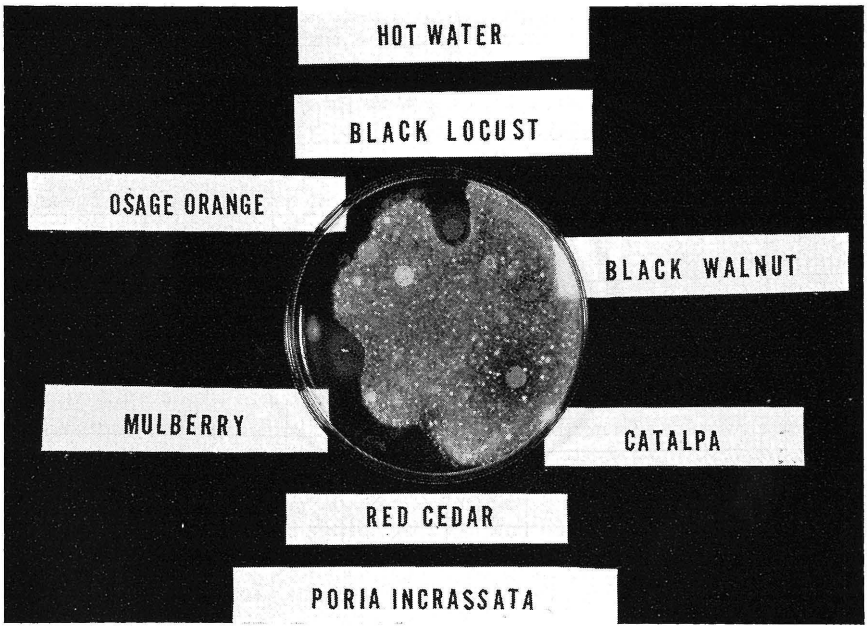


Fig. 4.—Hot water extracts of black locust and mulberry show inhibition of fungus.

TABLE 2.—Mean Percentages ± 1 Standard Deviation of Extractables Removed from Six Species by Seven Solvents

Species	(Spg.)	Cold H_2O	Hot H_2O	Benzene-Alcohol
Black locust	0.78 ± 0.02	3.85 ± 0.18	8.12 ± 0.15	7.32 ± 0.40
Black walnut	0.61 ± 0.05	4.82 ± 0.29	7.36 ± 0.29	7 28 ± 0.36
Catalpa	0.41 ± 0.02	5.17 ± 0.26	8.47 ± 0.29	6.97 ± 0.18
Eastern redcedar	0.50 ± 0.05	2.51 ± 0.27	5.11 ± 0.25	4.83 ± 0.10
Red mulberry	0.62 ± 0.03	6.03 ± 0.15	13.27 ± 0.86	11.53 ± 0.12
Osageorange	0.80 ± 0.02	5.86 ± 0.44	15.78 ± 0.99	14.61 ± 0.59

Species	Alcohol	Complete	E	AC
Black locust	7.37 ± 0.64	9.36 ± 0.22	0.26 ± 0.03	3.21 ± 0.12
Black walnut	7.40 ± 0.05	9.18 ± 0.20	0.12 ± 0.02	2.45 ± 0.11
Catalpa	7.89 ± 0.15	9.62 ± 0.35	0.09 ± 0.02	4.76 ± 0.14
Eastern redcedar	4.37 ± 0.20	5.61 ± 0.34	0.10 ± 0.02	1.91 ± 0.11
Red mulberry	11.77 ± 0.13	13.99 ± 0.87	0.61 ± 0.04	11.86 ± 0.31
Osageorange	14.53 ± 0.23	16.26 ± 0.17	0.75 ± 0.05	8.94 ± 0.24

effective as fungistats in two or more replications of the slurry series and supporting evidence from the other two techniques are considered to contain a compound or compounds which retard the six test fungi.

In order to determine if the inhibitory action was a result of inorganic elements, the extracted material was ashed and analyzed spectrographically. The results of the percentage of ash is shown in Table 2.

It is interesting to note that the hot water and complete extractions remove as much or more material from the wood than the other solvents, but do not necessarily inhibit the fungi. In the case of the complete series this may be due to a dilution effect. The individual extractions in complete series were diluted to 250 ml. in order to get as much material into the test solution as possible. The hot water may cause a chemical change of the extract due in part to the higher temperature. The hot water and complete series maintain temperature at 98° C. in the extraction chamber. Temperatures in the extraction chamber of the other series were: Acetone 56° C, Ether 32° C, Benzene Alcohol 80° C and Alcohol 78° C.

The efficiency of removing the inorganic ions from the wood can be seen in the table. Generally the hot water extraction removes more than any of the other extractions. The only exception to this is the complete extraction of black locust. In the complete series, the material

is first extracted with benzene:alcohol, and alcohol before the hot water extraction. Possibly these organics tie up the inorganics ion in such a way as to make it insoluble in the hot water. The efficiency of cold water to remove these inorganic ions is striking. In black locust, eastern redcedar and mulberry the ash content of the extracted material is essentially the same. In black locust the organic solvents used in these tests removed about the same amount of inorganics. Mulberry and osageorange have essentially the same amount of ash after extraction by benzene:alcohol and alcohol as the unextracted sample. These extractions proved to contain a fungistat. It would seem then that the compound responsible for inhibition in these two species is not an inorganic or metal-organic complex compound.

A spectrographic analysis of the ash of the unextracted samples indicated no differences between three classifications of natural durability. Table 3 includes the results of the analysis of the extracted samples based on 2 grams of wood.

Although striking differences in ash composition do exist between species there are no striking differences between durability classes. The exception to this would be the zinc in the black locust. In order to

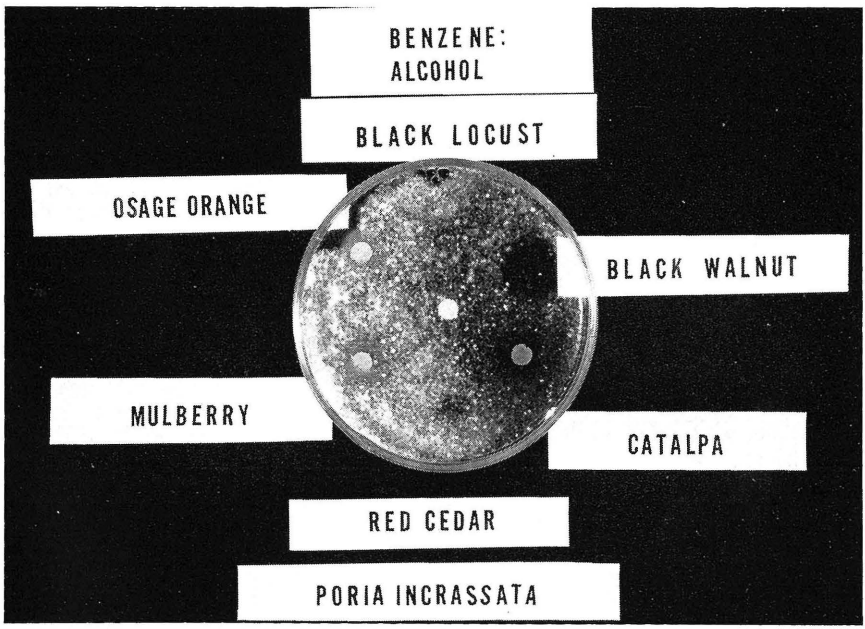


Fig. 5.—Alcohol:Benzene extracts of osageorange, black walnut, and mulberry show inhibition of fungus.

TABLE 3.—Weight of Elements in 2 Grams of Unextracted Materials (Grams × 10^{-6})

Durability Class (Yrs.)	Species	B	Mn	Mg	Pb	Fe	Al
(20-40)	Black locust	0.021	0.50	580.0	2.90	25.0	7.2
	Mulberry	0.296	0.28	287.1	1.50	38.0	5.5
	Osageorange	0.210	------	1148.0	3.10	20.0	10.1
(10-20)	Black walnut	0.480	0.44	249.6	14.40	37.4	9.8
	Catalpa	0.298	0.22	499.2	4.90	28.8	9.0
	E. redcedar	0.092	312.00	184.0	18.80	164.0	50.0
Less than 10	Corsican pine	0.190	64.90	996.8	0.97	101.8	57.7
	White ash	0.702	0.22	247.0	4.46	98.2	158.2
	River birch	0.191	73.10	925.8	2.42	95.1	81.0

Durability Class (Yrs.)	Species	Ca	Cu	Ag	Zn	Na	K
(20-40)	Black locust	3800	13.5	0.19	32.0	3.7	2600
	Mulberry	3762	4.4	0.32	9.8	14.3	13860
	Osageorange	6020	12.3	0.29	14.0	23.8	1092
(10-20)	Black walnut	9216	18.1	0.58	9.6	32.6	2342
	Catalpa	4128	14.4	0.24	9.6	11.0	2256
	E. redcedar	1760	14.0	0.15	0.7	120.0	400
Less than 10	Corsican pine	1341	6.5	0.05	6.7	10.8	581
	White ash	1506	11.3	0.02	6.1	2.3	1065
	River birch	1035	8.1	0.03	9.6	20.4	778

check this and other heavy metals which are known to be toxic to fungi, a spectrographic analysis of the extracted ash was made. All extracted samples were analyzed, however only those series which inhibited growth are included in this paper (Table 4). Results of the remaining extractions are on file.

The material removed must be considered to be in the extract, and therefore available to the fungi. If the elements analyzed are effective in prohibiting fungal growth one would expect the complete and hot water extraction series to respond systemically to their inclusion in the

TABLE 4.—Weight of Elements in Extracted Ashed Samples Based on Grams of Wood (Grams $\times 10^{-4}$)

Species	Extractions	B	Mn	Mg	Pb	Fe	Al
Black locust	Hot water	0.13	0.50	236	1.0	16.5	13.2
Black walnut	Benzene:Alcohol	0.20	0.37	205	2.7	7.2	3.5
Catalpa	Alcohol	0.12	0.23	390	2.6	23.0	9.0
Mulberry	Benzene:Alcohol	0.20	0.17	310	0.4	16.8	4.7
Mulberry	Alcohol	0.33	0.28	455	1.8	28.7	2.8
Mulberry	Complete	0.25	0.74	420	5.0	31.5	9.9
Osageorange	Benzene:Alcohol	0.14	*	767	0.9	6.8	6.0
Osageorange	Alcohol	0.16	*	741	1.3	39.2	5.6
Osageorange	Complete	0.11	*	553	0.9	9.7	7.0

Species	Extractions	Ca	Cu	Ag	Zn	Na	K
Black locust	Hot water	2090	3.4	0.09	31.9	4.0	962
Black walnut	Benzene:Alcohol	5704	3.1	0.26	6.2	6.6	967
Catalpa	Alcohol	2752	7.0	0.17	3.2	12.5	928
Mulberry	Benzene:Alcohol	3612	0.8	0.17	8.4	8.9	7728
Mulberry	Alcohol	5446	4.9	0.34	19.8	7.3	9108
Mulberry	Complete	3413	4.4	0.22	5.3	14.7	1995
Osageorange	Benzene:Alcohol	5074	2.6	0.19	11.8	6.3	435
Osageorange	Alcohol	5559	3.8	0.16	10.9	5.8	403
Osageorange	Complete	6018	2.4	0.18	9.7	5.1	169

*Less than 0.01.

extract. This is not the case. If the amount of a single element, such as copper, were responsible for inhibition of the test fungi, the complete extraction would be more effective. The elements which are in the extract are those which should aid fungal growth. Potassium and calcium are removed to a greater extent than the heavy metals. No major differences occur in inorganic composition between species or extractions or between durable and non-durable woods. It would seem then that site conditions should not play a large role in degree of durability from the standpoint of mineral ion concentration. The amount of heavy metal required to be systemic to fungi is probably toxic to the tree. These elements might be a catalyst for the compounding of organics which do act as fungistats.

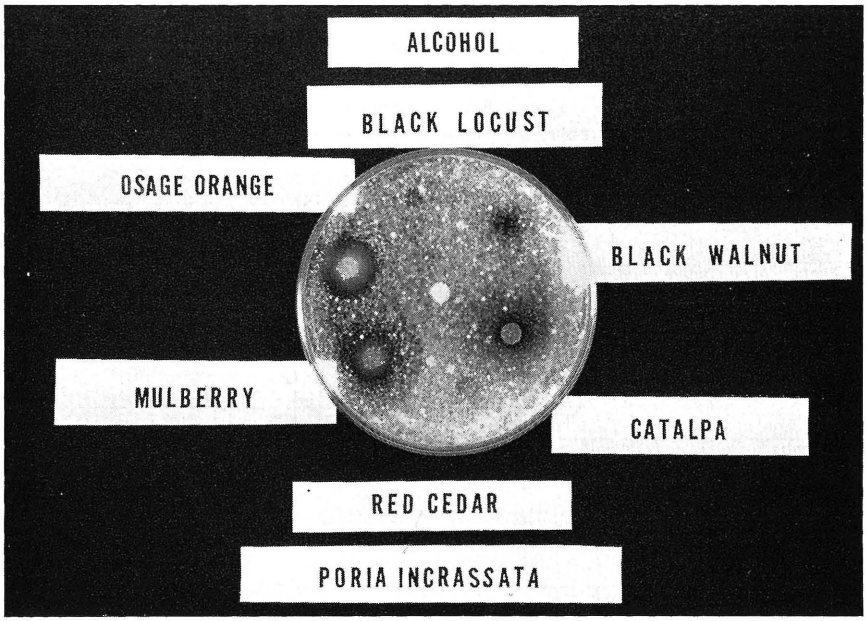


Fig. 6.—Alcohol extracts of osageorange, mulberry, and catalpa show inhibition of fungus.

CONCLUSIONS

From the results of the study the following conclusions may be drawn:

1. Fungi inhibiting compound or compounds can be extracted with
   (a) Alcohol from mulberry, osageorange and catalpa
   (b) Benzene:alcohol from mulberry, osageorange and black walnut
   (c) Complete extraction from mulberry and osageorange
   (d) Hot water from black locust.
2. No correlation could be found between twelve inorangic elements and the inhibition of test fungi.

This report covers the first phase of the study. The second phase now in progress is the chromatographic separation of the proven extracts. It will attempt to isolate the compound or compounds responsible for natural durability.